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# Genetics of seed flavonoid content and antioxidant activity in cowpea (*Vigna unguiculata* L. Walp.)

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ABSTRACT

Information about the type of gene action governing the inheritance of cowpea seed flavonoid content and antioxidant activity is prerequisite for starting an effective breeding program for developing improved varieties. For this purpose, half-diallel crosses among seven diverse parents were made. The homozygous parents and 21 $F_1$ hybrids were evaluated at Maroua in the Sudano-Sahelian zone of Cameroon using a randomized complete block design with three replicates. Flour samples produced from decorticated seeds were used for biochemical analysis. Analysis of variance showed significant differences ($P < 0.001$) among genotypes for the studied traits with ranges of 363.6–453.9 mg rutin equivalent per 100 g dry weight (DW) for total flavonoids, 13.38–30.73 mg ascorbic acid equivalent per 1 g DW for ferric iron reducing activity, 70.98–266.93 mg trolox equivalent per 100 g DW for 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, and 90.93–370.62 mg trolox equivalent per 100 g DW for 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity. Both additive and non-additive gene effects were significant in the genetic control of these traits, but dominance variance was greater than additive variance. The traits were mainly controlled by overdominance model suggesting a selection in the delayed generations. Broad- and narrow-sense heritability estimates varied from 0.90 to 0.99 and from 0.12 to 0.45, respectively. The variances due to both general and specific combining ability were highly significant for all studied traits. Recessive alleles had positive effects on DPPH and ABTS scavenging activities, whereas dominant alleles had positive effects on flavonoid content and ferric iron reducing activity. These results could help cowpea breeders to improve the antioxidant potential of cowpea seeds by appropriate selection.



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## 1. Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is a staple food that provides large amounts of proteins, calories, vitamins, and essential minerals for human nutrition in many countries [1–3]. It is consumed in central and western Africa mostly in the form of steamed paste cake (*koki* or *moinmoin*) and fritters (*kosai* or *akara*) [4,5]. Cowpeas are also used in the formulation of simple infant weaning foods that are relatively affordable for poor rural populations [6]. Cowpea seeds also contain phytochemicals that provide some health benefits to consumers.

Phenolic compounds such as flavonoids are plant secondary metabolites that play an important role in plant protection [7]. Although plant phenolics and specifically flavonoids have been classified as antinutrients, they are useful as natural antioxidants [8]. Levels of total flavonoids and antioxidant activity were correlated [9,10]. According to Enujiugha et al. [8] and Kumar et al. [11] epidemiological studies have revealed that the consumption of flavonoid-rich foods protects against human diseases associated with oxidative stress. Cowpea seeds are a good source of antioxidants, as reported recently [12–17]. Little information about the genetic variation in flavonoid content of cowpea seeds is available, except for the reports of Adeyemi and Olorunsanya [15], Apea-Bah et al. [17], and Salawu et al. [18]. To our knowledge, only Nzaramba et al. [12] and Noubissié et al. [16] have evaluated the genetic variation and inheritance of antioxidant activity by the DPPH method in cowpea. Both studies involved diallel and/or generation mean analysis involving four different pure lines to evaluate inheritance and other genetic effects. The importance of such studies is reinforced by the rejection of the synthetic antioxidants (butylated hydroxyanisole, butylated hydroxytoluene, and tertiary butylhydroxylquinone) by the consumers, in favor of natural antioxidants such as phenolic compounds [19,20].

As cowpeas are considered as poor persons' meat and are a principal source of protein for rural populations, it is important to evaluate the inheritance of their health-promoting traits for the development of elite genotypes. Diallel crossing is commonly adopted for evaluating parental lines for performance. It is an appropriate method for rapidly obtaining an overall picture of the genetic control of a trait in a set of inbred lines. This mating design has also been identified by Mather and Jinks [21] as a tool for evaluating genetic components underlying the inheritance of quantitative traits. To our knowledge, little information about the inheritance of antioxidants in various vegetables is available and no studies have evaluated the genetic components of total flavonoid content and antioxidant activity in cowpea in Cameroon's Sudano-Sahelian zone. The aims of this study were to evaluate total flavonoid content and antioxidant activity and elucidate their genetic control and inheritance in order to propose a suitable breeding strategy for improving the antioxidant potential of cowpea seeds.

## 2. Materials and methods

### 2.1. Experimental site

Field experiments were conducted from 2011 to 2013 at the IRAD (Institute of Agricultural Research for Development) farm of Giring (09°30′ N, 10°32′ E) in Maroua (Far North Cameroon). Giring is located in the Sudano-Sahelian zone with a ferruginous vertisol soil type. The soil is sandy clay with 8.2 mg $kg^{-1}$ of organic matter and pH of 5.65 [20]. Annual average rainfall ranges between 800 and 900 mm, with a 4-month rainy season from June to September. The mean annual temperature is 28 °C and the mean annual humidity is 40% [22].

### 2.2. Plant material and experimental design

Cowpea (*Vigna unguiculata* L. Walp.) seeds of 15 fully homozygous cultivars (two local landraces and 13 improved lines) were obtained from the IRAD in Maroua. Preliminary field screenings were performed during the rainy season in 2011 and 2012 to ensure the purity of the genotypes and evaluate their variation for flavonoid content and antioxidant potential. The experimental design was a randomized complete block design (RCBD) with three replications. Cowpea plants were grown in an experimental area of 384 $m^2$ (20.0 m length × 19.2 m width). The plot unit consisted of one row of 10 m length with an inter-row spacing of 80 cm. Three seeds were sown with an intra-row spacing of 25 cm and later thinned to one plant per hill. A safety and protection distance of 2 m surrounded the experimental field. At flowering stage, experimental plots were sprayed with a standard insecticide formulation, cypermethrin + dimethoate, at the rate of 30 g + 250 g a.i. $L^{-1}$ to control pod borers and other pests. Mature pods were progressively harvested and healthy seeds were carefully selected and kept in tagged envelopes.

### 2.3. Crossings

Seven genotypes (24-125B, B301, $BR_1$, CRSP, IT97K-573-1-1, Lori, and VYA) were selected as parents for diallel crossing on the basis of their genetic variability for these traits. Seeds of these parents were sown during the 2013 rainy season for crossing. At anthesis, plant-to-plant pollination of all possible crosses except reciprocals was made in 21 cross combinations following the 7 × 7 diallel crossing pattern. Each cross was tagged for easy identification, and at maturity, the $F_1$ seeds were harvested separately. The seven parental lines and the 21 $F_1$ hybrids obtained were planted in a RCBD with three replications during the 2014 rainy season. Plot unit size, spacing and treatments were as described above.

### 2.4. Biochemical analysis

A random sample of 0.25 g of flour prepared from seeds of each genotype following Phillips et al. [23] was used for the methanolic extraction of crude polyphenol compounds. Seeds were extracted with 15 mL of 70% methanol following Abdou Bouba et al. [24], and the extracts were used for all biochemical analyses.

*Total flavonoid content* was determined following Noudeh et al. [25] based on the flavonoid–aluminum complex with maximum absorption at 430 nm. A calibration curve was prepared with a 1 mg $mL^{-1}$ solution of rutin [26], and results were expressed as mg rutin equivalent on a dry basis.

*Ferric iron reducing activity (FIRA)* was evaluated by determining the ability of an antioxidant to reduce iron (III) to iron

(II) by the method of Oyaizu [27]. Absorbance was read at 700 nm and ascorbic acid was used as standard. FIRA was expressed as mg ascorbic acid equivalent per g of flour.

*DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity (FRSA)* was determined by the capacity of an antioxidant to trap a free radical or to donate a hydrogen atom, following Zhang and Hamauzu [28] with slight modifications. Trolox in varying concentrations was used as standard for the calibration curve, and the absorbance was read at 517 nm. The antioxidant activity of the extracts was expressed as mg trolox equivalent per 100 g dry weight (DW).

*ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] FRSA* was determined by ABTS radical cation decoloration following Re et al. [29]. Absorbances were read at 734 nm and stable values at room temperature during approximately 1 min were recorded. Trolox (0.625 g $L^{-1}$) at various concentrations (1.250 mmol, 0.833, 0.625, and 0.500 mmol $L^{-1}$) was used for the calibration curve. Results obtained were expressed as mg trolox equivalent per 100 g dry weight.

### 2.5. Statistical and genetic analyses

All biochemical analyses were performed in triplicate. To estimate genetic variation, data obtained from the 28 genotypes (parents and hybrids) were subjected to an analysis of variance (ANOVA) using STATGRAPHICS Plus 5.0 [30].

The genetic analysis was performed for a 7 × 7 half-diallel mating using the DIAL98 computer program [31]. Griffing's [32] method 2 (excluding reciprocal $F_1$ crosses) and model 1 (fixed effects) were used to estimate the general combining ability (GCA) of the lines and the specific combining ability (SCA) of crosses. GCA and SCA estimates of parents and hybrids, respectively, were obtained as

$$AGC_i = X_i – X, ASC_i = X_{ij} – X_i – X_j + X$$

where $X$ is the general mean of the population, $X_i$ is the mean of the hybrids from parent $i$, $X_j$ is the mean of the hybrids from parent $j$, and $X_{ij}$ is the value of the hybrid from parents $i$ and $j$.

Genetic parameters were estimated by Hayman's method [33]. Student's $t$ test was used to test the hypotheses that the GCA or SCA effects equal zero.

## 3. Results

### 3.1. Genotypic variability

The ANOVA for flavonoid content and antioxidant activities showed a significant difference ($P < 0.001$) between the various genotypes and hybrids studied (Table 1). There was also a significant effect ($P < 0.01$) of general and specific combining ability. The ratio $\sigma^2_{GCA}/\sigma^2_{SCA}$ showed values lower than 1, indicating the prevalence of non-additive gene effects in the genetic control of the studied traits.

### 3.2. Diallel analysis

The average values of parents (*per se*) and GCA of crosses revealed that the genotypes with highest flavonoids and ABTS-FRSA were $BR_1$ and B301, respectively (Table 2), whereas genotype Lori showed the highest FIRA and DPPH-FRSA. However, the genotypes with highest values sometimes showed the lowest GCA. This relationship was observed for $BR_1$ (the genotype with highest value), which presented a negative and significant ($P < 0.05$) GCA effect for flavonoid content. In contrast, the genotype 24–125B (with lowest value) presented a positive and significant ($P < 0.05$) GCA effect for flavonoid content (Table 2). Positive and significant combining ability is necessary for improving the antioxidant potential of seeds. The genotypes 24–125B and B301 showed positive and significant ($P < 0.05$) GCA effects for flavonoid content and FRSA (DPPH and ABTS), respectively. For FIRA, there were no positive and significant ($P < 0.05$) GCA effects.

For the combinations 24–125B × CRSP, B301 × IT97K-573-1-1, $BR_1$ × IT97K-573-1-1, CRSP × VYA, IT97K-573-1-1 × Lori, and Lori × VYA, positive and significant ($P < 0.05$) SCA effects were observed for flavonoid content (Table 3). The combination B301 × Lori presented a positive and significant ($P < 0.05$) SCA effect for all antioxidant activities.

The genetic parameters and their ratios were obtained by the graphical method of Hayman [33] (Table 4). Variances due to additive and non-additive effects were significant and indicated that all studied traits were under the control of an additive–dominance model. The $W_r$ (covariance values between the

**Table 1 – Mean squares obtained for total flavonoid and antioxidant activity for genotypes and combining ability in cowpea.**

| Source of variation | *df* | Mean squares | | | |
|---|---|---|---|---|---|
| | | Flavonoid | FIRA | DPPH | ABTS |
| Replication | 2 | 4541.94 | 0.2158991 | 106.60 | 860.73 |
| Genotypes | 27 | 9207.42*** | 307.921*** | 15,188.95*** | 27,843.18*** |
| Error | 54 | 1113.04 | 0.3932677 | 33.08 | 127.81 |
| GCA | 6 | 10,084.97** | 234.80** | 10,169.40** | 36,866.15** |
| SCA | 14 | 11,796.80** | 419.95** | 14,334.71** | 18,919.67** |
| Error (combining ability) | 40 | 1381.29 | 0.82 | 29.15 | 152.27 |
| $\sigma^2_{GCA}/\sigma^2_{SCA}$ | | 0.17 | 0.11 | 0.14 | 0.39 |

*df*, degrees of freedom; FIRA, ferric iron reducing activity; DPPH, DPPH free radical scavenging activity; ABTS, ABTS free radical scavenging activity; GCA, general combining ability; SCA, specific combining ability.

** Significance at the 0.01 probability level.

*** Significance at the and 0.001 probability level.

**Table 2 – *Per se* performance and general combining ability effects of parents for total flavonoid and antioxidant activity in cowpea.**

| Parents | *Per se* value and GCA effect | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Flavonoid [a] | | FIRA [b] | | DPPH [c] | | ABTS [d] | |
| | *Per se* | GCA | *Per se* | GCA | *Per se* | GCA | *Per se* | GCA |
| 24–125B ($P_1$) | 363.64 | 50.81** | 19.43 | 3.24 | 168.09 | −22.05 | 125.90 | 21.85 |
| B301 ($P_2$) | 400.78 | −10.24 | 17.23 | 2.09 | 165.06 | 45.70** | 370.62 | 97.42** |
| $BR_1$ ($P_3$) | 453.93 | −32.29* | 13.38 | −7.27** | 74.59 | −27.36* | 97.11 | 1.25 |
| CRSP ($P_4$) | 386.87 | 7.64 | 18.40 | 1.26 | 114.25 | −3.95 | 149.00 | −39.53 |
| IT97K-573-1-1 ($P_5$) | 436.43 | 5.07 | 17.22 | −3.81* | 95.03 | 20.99 | 178.12 | −20.68 |
| Lori ($P_6$) | 409.73 | −9.82 | 30.73 | 2.46 | 266.93 | −16.65 | 299.33 | −8.13 |
| VYA ($P_7$) | 367.73 | −11.17 | 23.50 | 2.03 | 70.98 | 3.32 | 90.93 | −52.17* |
| SE | | 9.80 | | 1.50 | | 9.84 | | 18.74 |

[a] Total flavonoid content in mg rutin equivalent in 100 g DW.
[b] FIRA, ferric iron reducing activity in mg ascorbic acid equivalent in 1 g DW.
[c] DPPH, DPPH free radical scavenging activity in mg trolox equivalent in 100 g DW.
[d] ABTS, ABTS free radical scavenging activity in mg trolox equivalent in 100 g DW; GCA, general combining ability; SE, standard error.
* Significant difference from zero at the 0.05 probability level.
** Significant difference from zero at the 0.01 probability level.

parents and their offspring in the *r*th array) on $V_r$ (variance values of the *r*th array) coefficients of regression were not significant for flavonoids (−0.29), FIRA (−0.11) and DPPH-FRSA (0.49), but ABTS-FRSA showed a positive and significant regression coefficient (0.84). However, it is noteworthy that the additive (*D*) and environmental (*E*) variances were lower than the two components of the dominance variance ($H_1$ and $H_2$). In addition, the values of average degree of dominance $[(H_1/D)^{1/2}]$ above 1 showed overdominance and those of proportion of dominant genes $[kd/(kd + kr)]$ confirmed the prevalence of dominance over additive. The positive sign of the term *h* indicated that most alleles were dominant for flavonoid levels

**Table 3 – Mean values and estimates of specific combining ability effects of crosses for total flavonoid and antioxidant activity in cowpea.**

| Crosses | Flavonoid [a] | | FIRA [b] | | DPPH [c] | | ABTS [d] | |
|---|---|---|---|---|---|---|---|---|
| | Mean value | SCA | Mean value | SCA | Mean value | SCA | Mean value | SCA |
| $P_1 \times P_2$ | 462.70 | 18.23 | 20.33 | −6.98* | 27.64 | −12.10 | 178.83 | −17.88 |
| $P_1 \times P_3$ | 430.98 | −49.29** | 14.23 | 15.33*** | 47.96 | 47.66** | 5.57 | 11.38 |
| $P_1 \times P_4$ | 305.96 | 102.50*** | 25.59 | −11.87** | 210.79 | 2.69 | 73.86 | 16.91 |
| $P_1 \times P_5$ | 437.15 | 2.05 | 21.88 | −4.47 | 75.93 | −45.53* | 213.75 | 51.59* |
| $P_1 \times P_6$ | 460.25 | 13.23 | 23.70 | 11.96** | 10.05 | −12.56 | 63.29 | −108.65*** |
| $P_1 \times P_7$ | 357.00 | −86.72*** | 27.45 | −3.97 | 177.98 | 19.85 | 117.81 | 46.66* |
| $P_2 \times P_3$ | 374.02 | 18.38 | 36.68 | 1.10 | 72.48 | −18.96 | 160.55 | 75.39** |
| $P_2 \times P_4$ | 565.73 | −21.78 | 18.00 | −6.33* | 50.93 | −39.49* | 125.31 | −47.83* |
| $P_2 \times P_5$ | 463.58 | 37.55* | 23.72 | −1.78 | 85.79 | −65.00** | 227.46 | 10.93 |
| $P_2 \times P_6$ | 459.01 | −53.17** | 43.03 | 11.23** | 22.98 | 155.88*** | 31.14 | 136.67*** |
| $P_2 \times P_7$ | 357.00 | 0.79 | 27.45 | 2.76 | 75.36 | −20.34 | 117.81 | −157.28*** |
| $P_3 \times P_4$ | 376.62 | −3.52 | 21.89 | 2.53 | 34.10 | −8.82 | 92.24 | 4.44 |
| $P_3 \times P_5$ | 380.63 | 53.41** | 21.30 | −0.06 | 73.61 | −19.90 | 300.13 | −101.08*** |
| $P_3 \times P_6$ | 317.98 | −44.70** | 19.21 | −1.36 | 46.23 | 16.01 | 86.64 | −32.56 |
| $P_3 \times P_7$ | 387.05 | 25.72 | 2.61 | −17.53*** | 34.21 | −15.98 | 117.60 | 42.44* |
| $P_4 \times P_5$ | 367.07 | −111.53*** | 22.40 | 2.77 | 76.50 | 119.52*** | 136.14 | 7.99 |
| $P_4 \times P_6$ | 381.03 | −21.58 | 17.35 | −11.74** | 2.75 | −50.88** | 105.12 | 26.70 |
| $P_4 \times P_7$ | 457.16 | 55.90** | 53.30 | 24.64*** | 50.58 | −23.02 | 26.17 | −8.21 |
| $P_5 \times P_6$ | 331.56 | 60.22** | 41.16 | −0.32 | 259.17 | −68.52** | 352.04 | −33.99 |
| $P_5 \times P_7$ | 384.16 | −41.69** | 32.25 | 3.86 | 102.92 | 79.43*** | 14.05 | 64.57** |
| $P_6 \times P_7$ | 429.81 | 46.01** | 20.10 | −9.76** | 20.97 | −39.94* | 77.61 | 11.83 |
| SE | | 11.45 | | 2.16 | | 12.62 | | 14.50 |

[a] Total flavonoid content in mg rutin equivalent in 100 g DW.
[b] FIRA, ferric iron reducing activity in mg ascorbic acid equivalent in 1 g DW.
[c] DPPH, DPPH free radical scavenging activity in mg trolox equivalent in 100 g DW.
[d] ABTS, ABTS free radical scavenging activity in mg trolox equivalent in 100 g DW; SCA, specific combining ability; SE, standard error.
* Significant difference from zero at the 0.05 probability level.
** Significant difference from zero at the 0.01 probability level.
*** Significant difference from zero at the 0.001 probability level.

**Table 4 – Some genetic parameters and ratios obtained from a 7 × 7 half diallel in cowpea.**

| Genetic parameter and ratio | Flavonoid | FIRA | DPPH | ABTS |
|---|---|---|---|---|
| $D$ | 738.97 | 32.40 | 4865.51 | 11,445.95 |
| $H_1$ | 14,929.54 | 421.51 | 22,410.91 | 24,808.67 |
| $H_2$ | 12,168.99 | 401.09 | 18,259.90 | 21,532.84 |
| $F$ | 2604.25 | 7.29 | 7355.52 | 5751.39 |
| $E$ | 400.06 | 0.26 | 10.40 | 44.75 |
| $hh$ | −168.46 | 84.97 | 11,349.84 | 11,000.37 |
| $(H_1/D)^{1/2}$ | 4.50 | 3.61 | 2.15 | 1.472 |
| kd/(kd + kr) | 0.70 | 0.52 | 0.68 | 0.59 |
| $hh/H_2$ | −0.02 | 0.25 | 0.73 | 0.60 |
| $h$ | 3.67 | 9.22 | −106.56 | −104.98 |
| $H_2/4H_1$ (±uv) | 0.20 | 0.24 | 0.20 | 0.22 |
| $h^2$ | 0.90 | 0.98 | 0.97 | 0.99 |
| $h^2_n$ | 0.12 | 0.19 | 0.15 | 0.45 |
| Regression ($V_r$, $W_r$) | $-0.29V_r + 663.3$ | $-0.11V_r + 26.6$ | $0.49V_r$–1864.4 | $0.84V_r$–2127.0 |
| $r$ ($Pr$, $W_r + V_r$) | $-0.18^{ns}$ | $0.34^{ns}$ | $0.91^{**}$ | $0.86^{*}$ |
| Regression ($Pr$, $V_r + W_r$) | $-9.02Pr + 6612.1$ | $3.58Pr + 56.1$ | $93.05Pr$–7072.3 | $91.22Pr$–5129.5 |

FIRA, ferric iron reducing activity; DPPH, DPPH free radical scavenging activity; ABTS, ABTS free radical scavenging activity; $D$, additive variance; $H_1$, $H_2$, dominance variances; $F$, product of additive by dominance effects; $E$, environmental variance; $hh$, square of difference of parents *versus* whole diallel; $(H_1/D)^{1/2}$, average degree of dominance; $kd/(kd + kr)$, proportion of dominant genes; $hh/H_2$, number of effective factors; h, average direction of dominance; $H_2/4H_1$ (±uv), balance of positive and negative alleles; $h^2$, $h^2_n$, Broad- and narrow-sense heritability; $V_r$: variance values of the $r$th array; $W_r$: covariance values between the parents and their offspring in the $r$th array; $r$ ($Pr$, $W_r + V_r$), correlation between the degree of dominance of the parents ($W_r + V_r$) and the parental value ($Pr$); ns, not significant.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

and FIRA but not for DPPH-FRSA and ABTS-FRSA. This trend is confirmed by the value of balance of positive and negative alleles ($H_2/4H_1$) below 0.25 (the theoretical maximum), indicating that dominant and recessive alleles were unevenly distributed among the parents. Overall, the correlation coefficient between the degree of dominance of the parents ($W_r + V_r$) and the parental value ($Pr$), was positive and significant for DPPH-FRSA and ABTS-FRSA, but not for FIRA, which showed a positive and nonsignificant coefficient. Recessive alleles thus had a positive effect on DPPH-FRSA and ABTS-FRSA. For flavonoid content, a negative and nonsignificant correlation coefficient was observed; dominant alleles had a positive effect on this trait and a slightly positive effect on FIRA. All the traits studied were highly heritable ($h^2$ = 0.90–0.99), with the variance due to genetic interactions greater than environmental variance. Low (0.12–0.45) narrow-sense heritability ($h^2_n$) values were observed, confirming the superiority of dominance over additivity.

## 4. Discussion

The present results show broad genetic variability for flavonoid content and antioxidant potential in cowpea seeds. This variability can be exploited in selection for the development of new varieties with high nutritive value. Qualitative tests performed by Adeyemi and Olorunsanya [15] showed that three of four cowpea varieties tested contained flavonoids. According to Salawu et al. [18], the total flavonoid content in cowpea ranged from 0.95 to 0.36 mg quercetin equivalents $g^{-1}$. However, a high flavonoid content (12,226 μg $g^{-1}$ DW) was observed in cowpea flour for which the major flavonoids subclasses were flavonols and flavan-3-ols [17]. Recent studies by El-Mergawi and Taie [34] and Fouad and Rehab [35] in faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medik.) gave approximately similar flavonoid contents to those obtained in the present study. Several other studies of faba beans as well as African yam bean (*Sphenostylis stenocarpa* Hochst. Ex A. Rich), *Acacia* species, chickpea (*Cicer arietinum* L.), and lupine (*Lupinus albus* L.) [8,36–39] revealed low values for total flavonoid content. By contrast, a high flavonoid content (20.9 ± 0.8 mg catechin equivalent in 1 g extract) was obtained for pigeon pea (*Cajanus cajan* L.) in a methanolic extract of seed coat and whole seed extract [40]. Despite using the same standard, like catechin, rutin, quercetin, kaempférol, *etc.*, we observed a large variation in results. This variation may have been due to the measuring methods used, processing applied, storage conditions and duration, quality of the standard used, and genetic factors [41].

Cultivars with darker seed coat show higher total flavonoid content than white cultivars in two species [18,37]. Positive relationships between dark hull color and antioxidant activity in legume seeds have been reported [42,43]. Whole seeds of pigeon pea showed higher antioxidant properties than cooked whole pod [40]. The antioxidants may be located mainly in the hull or seed coat and be removed by leaching. In general, antioxidant activity of an extract cannot be predicted on the basis only of its total phenolic content [24].

Antiradical activity is determined by two methods, the first generally applied to cereals using the DPPH radical and the second, using the ABTS radical, applied for simple compounds and complex mixtures [44,45]. The ABTS radical used is a nonphysiological radical source not found in mammals [46]. Probably for this reason, Ba et al. [47] observed that values obtained by ABTS were greater than those found by DPPH. All cowpea lines used in this study showed high antioxidant activity according to DPPH, ABTS, and ferric reducing power assays. All genotypes except 24–125B and VYA showed values of ABTS-FRSA greater than those for DPPH-FRSA, in agreement

with the findings of Ba et al. [47]. High biological and antioxidant activities have been found previously in cowpea seeds and other legume species [8,17,36,39,43].

In the present study, the traits studied were controlled by both additive and non-additive gene effects with a preponderance of non-additive gene effects. The traits studied were thus controlled mainly by dominant genes and were not strongly affected by environment. Indeed, high dominance variance, low narrow-sense heritability, and an average degree of dominance greater than unity were estimated. The same conclusion was reported by Karmakar et al. [48] for antioxidants in fresh fruits of ridge gourd (*Luffa acutangula* Roxb.). Hence, attention must be focused on hybrid breeding to produce flavonoid- and antioxidant-rich genotypes of cowpea seeds for flavonoids and FIRA, but not for DPPH-FRSA and ABTS-FRSA, where judicious selection of superior parents would be effective. The superiority of hybrids over parents may have been due to the presence of heterozygous loci in the hybrids, leading to heterosis [48]. These traits seemed to be controlled by partial dominance. In this case, the selection of elite parents would be an efficient method for breeding varieties rich in these two antioxidant activities. The negative values for DPPH-FRSA and ABTS-FRSA were in agreement with the findings of Karmakar et al. [48] for antioxidant potential, indicating a prevalence of recessive alleles for these traits in parents. The average degree of dominance greater than unity for all of the studied traits revealed the presence of overdominance in the genetic control of traits. Karmakar et al. [48] identified 2–3 groups of genes that controlled the traits and exhibited dominance for DPPH- and ABTS radical scavenging activity. Our study indicated about one group of genes, perhaps because of the prevalence of epistasis for these traits. Moreover, the additive-dominant model was not established in this study, given the nonsignificant regression coefficients ($V_r$, $W_r$) for flavonoids (−0.29), FIRA (−0.11), and DPPH-FRSA (0.49). Epistasis could be involved in the genetic control of these traits. Only ABTS-FRSA obeyed the additive-dominant model without epistasis. Additivity, dominance, and epistasis have been reported to be involved in the genetic control of phenolics and antioxidant activity [10,14]. All traits studied were highly heritable, but narrow-sense heritability values lower than 50% showed once again that non-additive gene action played a major role in the inheritance of flavonoids and antioxidant activities.

From the present study, the prevalence of non-additive gene action suggests the adoption of a hybrid breeding strategy. According to Nzaramba et al. [12], breeding for high antioxidant activity in cowpea is achievable by use of dark-colored genotypes. In fact, these authors established a positive correlation between testa color and antioxidant activity.

## 5. Conclusions

Wide genetic variability was found for total flavonoid content and antioxidant activity in cowpea seeds. These traits were highly heritable and controlled mainly by non-additive gene effects. Recessive alleles exerted a positive effect on DPPH-FRSA and ABTS-FRSA and a negative effect on flavonoids and FIRA. Thus, recurrent selection schemes will be a relevant breeding strategy for the improvement of the antioxidant potential of cowpea seed in the Sudano-Sahelian zone.

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